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PRINCIPAL INVESTIGATOR: Andrea M. Allan, Ph.D.

CONTRACTING ORGANIZATION: University of New Mexico
Albuquerque, New Mexico 87131-5041

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6. AUTHOR(S):
Andrea M. Allan, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of New Mexico
Albuquerque, New Mexico 87131-5041

E-Mail: aallan@salud.unm.edu

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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
Alcohol use has been identified as an important factor in aggressive, or violent, behavior in humans. Alcohol not only increases the incidence but also the severity of violent attacks. Several clinical studies have reported the observation that highly aggressive individuals display a serotonin-deficient trait. A number of studies indicate that the $5HT_3$ receptor system mediates alcohol consumption and the subjective effects of alcohol. The $5HT_3$ receptor is unique in the serotonin receptor family in that it is a cation channel and modulates the release of a number of other neurotransmitters, including GABA and dopamine. Thus, the 5-HT_3 receptor is likely to play a critical role influencing alcohol consumption, which appears to involve dopamine and influencing both natural and alcohol-heightened aggression through the $GABA_A$ receptor system. We have developed a 5-HT_3 receptor over-expressing mouse to study the role of this receptor in alcohol drinking and aggression. We hypothesize that $5HT_3$ receptor over-expression decreases alcohol preference and aggressive activity through a $5HT_3$ receptor sensitive mechanism increasing the release of GABA. We will evaluate the transgene on 3 different inbred strains (C57, DBA, 129) who differ in alcohol preference and alcohol-induced aggression. We will measure the impact of $5HT_3$ receptor over-expression on alcohol preference in a two-bottle choice design and aggression using intruder-aggression test. We will test the ability of $5HT_3$ receptor antagonists to block and GABA receptor agonist to mimic the phenotypic effects of over-expression. These mice should prove useful in testing hypothesis regarding the role 5-HT_3 receptors play in alcohol abuse and alcohol-heightened aggression.

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Introduction

Alcohol use has been identified as an important factor in aggressive, or violent, behavior in humans. In its 10th Special Report to the U.S. Congress on Alcohol and Health (June 2000), the Public Health Service stated that one in four victims of violent crime described their attacker as having consumed alcohol prior to committing the crime. When the victim was a current or former intimate partner, the incidence increased to two out of three offenders having been drinking prior to the attack. Alcohol not only increases the incidence but also the severity of the attack. While these statistics may be correlational, a causal relationship between alcohol use and physical violence needs to be explored.

Experimentally, aggression in mice can be brought about by a isolating male for some time, then, subsequently, to introduce a group-housed male to the isolated male's home cage (*isolation-induced aggression or intruder-aggression*), with heightened attacks seen when the resident mouse is an actively breeding male (Malick, 1979; Miczek, 1999). Many environmental and experiential variables factor into aggressive behavior, but the administration of alcohol is an important societal variable in aggressive behavior (Miczek et al., 1998). Several lines of research on the neurochemical basis for aggression have implicated the serotonin system (Korte et al., 1996; Fish et al., 1999; DeAlmedia et al., in press). Alterations in the serotonin system, using knock out technology, have produced lines of mice that display altered aggressive behavior (Saudou et al., 1994; Cases et al., 1995). Pharmacologic studies have implicated the involvement of 5-HT_{1a} (deBoer et al., 2000), 5-HT_{1b} (Fish et al., 1999) and 5-HT_3 (Rudissaar et al., 1999) receptors. Several clinical studies have reported the observation that highly aggressive individuals display a serotonin-deficient trait, as measured by either low levels of cerebral spinal fluid (CSF) 5-hydoxyindole acetic acid (5-HIAA) or a flattened prolactin response to serotonin activation (Brown et al., 1982; Linnoila et al., 1983; Coccaro et al., 1990, Mann, 1999). While these data provide support for a role for serotonin in aggression, there is strong support for gamma amino butyric acid (GABA) as well (Soderpan and Svensson, 1999; Glaysheva et al., 1998; Guillot et al., 1998; Navarro and Pedraza, 1996; Miczek et al., 1995; 1994,1993; Weerts et al., 1993).

Besides the suggestion that serotonin plays a role in aggression, several lines of research have suggested a role for serotonin in the regulation of alcohol consumption. A number of studies indicate that the $5HT_3$ receptor system mediates alcohol consumption and the subjective effects of alcohol. $5HT_3$ receptor antagonists decrease alcohol intake in laboratory animals (Jankowska et al., 1995, Jankowska et al. 1994, Tomkins et al. 1995, Knapp and Pohorecky 1992, Hodge et al. 1993) and humans (Johnson et al., 1993; Sellers et al., 1994). In addition, $5HT_3$ receptor antagonists increase the subjective feeling of alcohol intoxication in humans (Swift et al., 1996), suggesting that $5HT_3$ receptors are involved in alcohol sensitivity. Lovinger and his colleagues have shown that alcohol directly potentiates the $5HT_3$ receptor (Lovinger, 1991; Lovinger and White, 1991; Lovinger and Zhou, 1994). The $5HT_3$ receptor is unique in the serotonin receptor family in that it is a cation channel and modulates the release of a number of other neurotransmitters, including GABA and dopamine. Thus, the 5-HT_3 receptor is likely to play a critical role influencing alcohol consumption, which appears to involve dopamine and influencing both natural and alcohol-heightened aggression through the $GABA_A$ receptor system.

We hypothesized that over-expression of 5-HT_3 receptors decreases alcohol consumption because the presence of an increased number of 5-HT_3 receptors increased the potentiation of dopamine release at lower alcohol concentrations. Thus, the animal requires less alcohol to obtain the same behavioral effect. Alternatively, it is possible that the over-expression of 5-HT_3 receptors increases the release of gamma aminobutyric acid (GABA), an inhibitory neurotransmitter. GABA plays an important role in the inhibitory circuit from the prefrontal cortex to the amygdala and ventral striatal areas. This pathway is thought to play a role in moderating impulsive and compulsive behaviors. Thus, the lower level of alcohol consumption seen in the 5-HT_3 receptor over-expressing mice may be the result of increased inhibitory control over alcohol consumption. That is, these transgenic mice know "when to say when". To add support for this hypothesis, we found that the 5-HT_3 receptor over-expressing mice fail to behave aggressively in an intruder aggression test. The wild type mice clearly displayed high levels of aggression by attacking the intruder within seconds of introduction and continuing the attacks until the test was halted. This lower level of aggression in the 5-HT_3 receptor over-expressing mice may be due to an increase in GABA activity. The proposed studies will explore the neurochemical relationship between the lower alcohol consumption and lower levels of aggression seen in 5-HT_3 receptor over-expressing transgenic mice. The first goal will examine the impact of $5HT_3$ receptor over-expression on alcohol preference using a two-bottle free choice test. The second goal will examine the impact of $5HT_3$ receptor over-expression on natural aggressive behavior. Lastly, the impact of $5HT_3$ receptor over-expression on alcohol-heightened aggressive behavior will be measured.

Body

Progress made towards our goals are as follows: Breeding onto the three different backgrounds was initiated. Generations N1, N3 and N5 are the tested generations planned. We have completed generation N1 testing and have begun generation N3 testing.

Lower Alcohol Drinking Phenotype Conserved On Various Genotypic Backgrounds in the first generation of cross breeding

Reduced alcohol preference as a result of $5HT_3$ receptor over-expression was conserved even when expressed on a genetic background known for high alcohol preference, C57BL/6J (Figure 1). Using a two-bottle free choice test, C57BL/6J mice drank an average of 14 g/kg/day, while the Line 13 x C57BL cross transgenic mice drank an average of 8 g/kg/day, while the 129 mice drank an average of 8 g/kg/day the line13X129 drank 3.75 g/kg/day. The transgene did not influence the DBA drinking which remained below 1g/kg/day.. **Thus, the presence of the transgene was able to influence alcohol preference on a different genetic background even though these mice are heterozygous for the transgene.**

As depicted below in Figure 1 below, the influence on the transgene to reduce alcohol drinking in high alcohol consuming strains is maintained on the first generation background. However, preliminary data from the N3 generation suggests that this influence is lost, at least on the C57BL/6 background. This data is still being analyzed and evaluated.

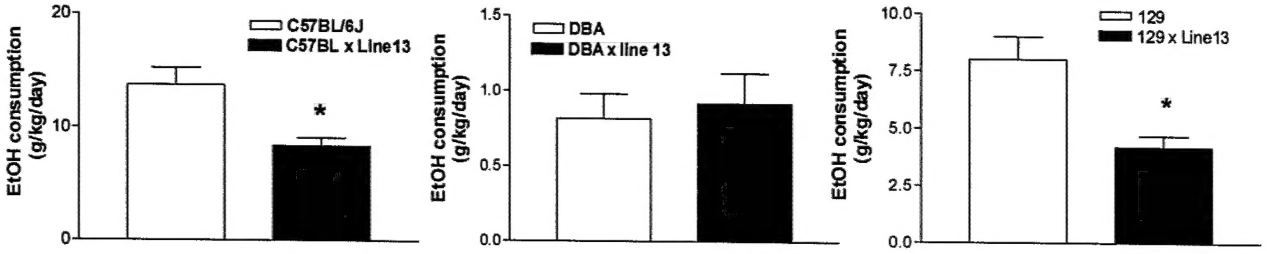


Figure 1 *Ethanol consumption averaged over 10 days in a two bottle free choice test in C57BL/6 xLine13 cross compared to C57BL/6 mice (left panel), DBA/2J vs. DBA/2J x line 13 cross mice (center panel) and 129/J xLine13 cross compared to 129/J mice (right panel). Data are mean ± SEM, n=10. Asterisk indicates significance at p<0.05.*

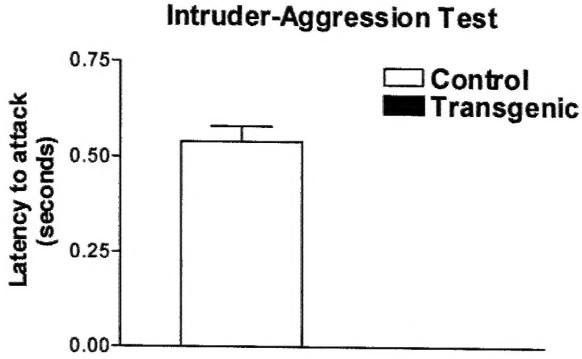


Figure 2: *Intruder aggression test. Latency to first attack in seconds. Data are mean +/-SEM, n=5 mice. Male group housed A/J mice served as intruders. Residents housed singly 2 week prior. Testing was performed in the resident's home cage.* ***Transgenic mice failed to attack during the 10 minute test period, thus no data is presented for them.***

5-HT$_3$ Receptor Over-Expression Significantly Lowers Intruder Aggression

Using a standard intruder test, we evaluated the level of aggressive behavior in the transgenic 5-HT$_3$ receptor over-expressing mice. Resident mice were isolated for

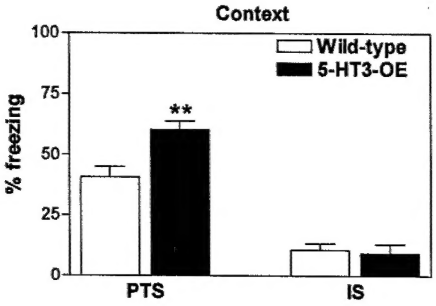
one week prior to the testing. A/J male group housed mice were used as the intruders. The intruder mouse was placed bite. If no biting took place the test was terminated after 5 minutes. Thus, the longest the test was run would have been 10 minutes. In this way we avoid any bias against aggressive behavior that may begin later in the testing period but yet can still be very intense in nature. Further measurements of latency alone may distort the measures of actual fighting once it is initiated.

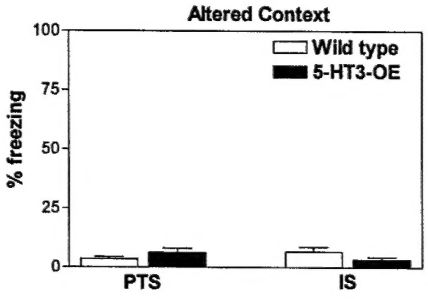
Transgenic mice failed to engage in biting or other forms of attack (Figure 2). These were single tests and these were not breeding males. Repeated testing and breeding male tests reveal a different picture. In repeated engagements, transgenic mice did attack the intruder with a frequency nearly indistinguishable form that of the wild type males. We have measured alcohol-heightened aggression in preliminary tests. While the transgenics do show an increase in aggressive behavior with alcohol, aggression is still significantly lower than in the control mice, with an average of 3.5 attack bits for transgenics and 11 attack bites for controls after 1 g/kg alcohol injected i.p. 10 minutes prior to testing. Data on aggression following free access alcohol are preliminary and additional animals need to be studied. Further we are measuring the effect of repeated encounters in the resident's cage.

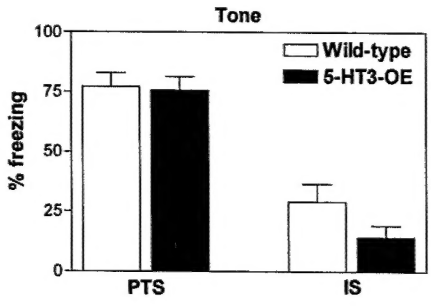
5-HT$_3$ Receptor Over-Expression Significantly Improves Learning

As an additional measure of impulse control we have been examining learning using a fear conditioned freezing measure with these lines. Fear conditioning is a classical Pavlovian learning measure where the mouse learns to anticipate a foot shock through the association of a preceding tone and the learning context itself. Learning is indicated by freezing. Over-expression of the 5HT3 receptor significantly improves learning in this system (see figure below). These data suggest that the over-expression of the 5HT3 receptor permits better stimuli association and information processing. Additionally, these results indicate that over-expression of the 5-HT3 receptor in mouse forebrain results in enhanced hippocampal-dependent learning and attention.

Freezing scores for 5-HT3 receptor transgenic and control mice







Key Research Accomplishments

- Initiate moving the transgene on to the three inbred strain backgrounds through breeding.
- Test the two bottle choice drinking on the wild type and transgenic N1 generations of the three strains.
- Test initial and repeated aggressive behavior on the wild type and transgenic N1 strains.
- Characterize unique learning and stress responding in the mice which impacts consumption and aggression.
- Began alcohol induced aggressive behavior testing on the N1 strains.
- Breed forward to the N3 generations.

Reportable Outcomes

Harrell, A.V., Caldwell, K.K, **Allan, A.M**. "Transgenic mice over-expressing the 5-HT3 receptor have enhanced learning in latent inhibition and contextual fear conditioning paradigms" Society for Neurosciences, 2001

Carta, M. **Allan, A.M**. Partridge, L.D., Valenzuela, C.F. Effect of cocaine on 5-HT3 receptor mediated currents in hippocampal neurons from transgenic mice over expressing the receptor. Society for Neurosciences, 2001

Allan, AM, Chynoweth, J and Caldwell,K K "A Moderate exposure fetal alcohol mouse model using saccharin fading technique." Research Society on Alcoholism 26:531, 2002

Carta, M. **Allan, A.M**. Partridge, L.D., Valenzuela, C.F. Effect of cocaine on 5-HT3 receptor mediated currents in hippocampal neurons from transgenic mice over expressing the receptor. Submitted to Eur. J. Pharmacology, 2002

Harrell, A. V. and **Allan, A.M**. Improvements in Hippocampal-Dependent Learning and Decremental Attention in 5-HT3 Receptor Over-Expressing Mice. Submitted to Learning & Memory, 2002

Conclusions

At least a generation 1 of the cross breeding the original impact of the transgene appears stable. However, as the background becomes more inbred the influence seems to be diluted. Further, the impact of the transgene on reducing aggressive behavior is upon initial exposure to the intruder but is overwhelmed but repeated presentations of the threat. This suggests a learned component or plasticity involved in aggressive responding that has not been noted in the literature at this time. Can aggressive responding be interfered with pharmacologically? We plan to look into these questions in future studies.

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